Cytotoxic Activity of Marine Algae and a Cytotoxic Principle of the Brown Alga *Sargassum tortile*

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Partition fractions of hexane, CCl_4 and $CHCl_3$ from methanolic extracts of marine algae were each examined for cytotoxic activities against cultured P-388 lymphocytic leukemia cells. Cytotoxic activities were found for partition fractions of 21 species of seaweed. Bioactivity-guided fractionation of the CCl_4 partition fraction from *Sargassum tortile*, exhibiting the most prominent activity, afforded dihydroxysargaquinone (1) and sargatriol (2) previously isolated from this alga. The former was evaluated as a cytotoxic principle, and the latter, showing moderate activity, was suggested to be an artifact derived from 1 during the isolation procedure.

Keywords marine alga; *Sargassum tortile*; cytotoxicity; dihydroxysargaquinone; sargatriol; benzoquinone; chromene

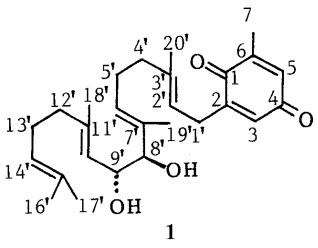
Previously it was reported that water extracts from some seaweeds showed tumor-inhibiting effects against Sarcoma-180 implanted subcutaneously in mice, or against Ehrlich ascites carcinoma in mice, and the tumor-inhibiting principle was a polysaccharide, whose tumor-inhibiting effect was suggested to be mainly host-mediated.[1] We initiated a survey of marine algae for antineoplastic and/or cytotoxic constituents other than polysaccharides. This paper describes the screening results of various seaweeds in the P-388 lymphocytic leukemia test system *in vitro*, in which a cytotoxic principle was found from the brown alga *Sargassum tortile* C. AGARDH (yoremoku in Japanese) (Sargassaceae).

Twenty-four species of fresh seaweed, collected along the coast of Tanabe Bay in Japan in May 1985, were extracted with MeOH, and a solution of each extract in aqueous MeOH was partitioned successively with hexane, CCl_4 and $CHCl_3$. The resulting fractions of each seaweed were tested for their growth-inhibitory activity on P-388 cultured cells. As a result, some partition fractions of 21 species of seaweed were shown to exhibit growth inhibition against P-388 cells, and the most prominent activity was found to be located in the CCl_4 partition fraction of *S. tortile* (Table I). This result led us to investigate the cytotoxic principle of *S. tortile*.

The CCl_4 partition fraction from the MeOH extract of *S. tortile* was purified by bioassay-directed fractionation employing Sephadex LH-20 and silica gel column chromatographies to afford dihydroxysargaquinone (**1**) and sargatriol (**2**), already isolated from this alga (Chart 1).[2] These compounds were identified by comparison of their spectral data with the reported values.[2]

For sargatriol (**2**), Kikuchi *et al.* proposed the C_2-α-methyl structure (**2a**) on the basis of a Cotton effect in the 265—275 nm region of circular dichroism (CD).[2a] The CD data of **2** obtained herein was consistent with the published values.[2a] However, in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **2**, each of C-3, C-10, C-4′ and C-16′ appeared as two signals (*ca.* 1 : 1) as shown in Table II. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **2** also exhibited signals due to an epimeric mixture (see Experimental). The same feature was observed in the ^{1}H- and ^{13}C-NMR spectrum of the methyl ether (**3**) derived from **2**. In addition, optical rotation and CD curves were not observed in the aldehyde (**4**) derived by oxidation of **3** with a chromium trioxide–pyridine complex. Furthermore, cyclization of **1** with hot pyridine afforded a chromene derivative identical to **2**. The sum of this evidence shows that sargatriol, obtained herein and previously,[2a] is a mixture of two epimers with respect to C-2. In addition to this evidence, the fact that crude dihydroxysargaquinone (**1**) converted into **2** on standing in a bit of MeOH at room temperature for about one month suggests the possibility that **2** is an artifact of the isolation procedure. The 265 nm CD value of **2**, identical to the published one,[2a] is one-tenth that of general chiral styrenes.[3] This weak Cotton effect is considered to appear as the sum of Cotton effects of two C-2 epimers, in which the signs are opposite and the absolute values are different due to the prenyl side-chain with asymmetric centers.

The absolute configuration of **1** has not yet been established. The above-mentioned transformation of **1** to **2** demonstrates that **1** has an *R* configuration at both



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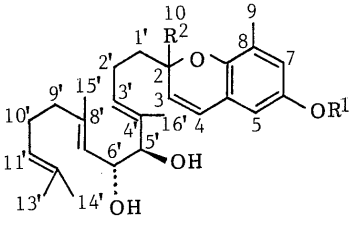

2 : R^1=H, R^2=CH_3 ; C_2 epimeric mixture

2a : R^1=H, R^2=β-CH_3

3 : R^1=R^2=CH_3, C_2 epimeric mixture

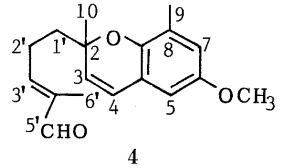

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Chart 1

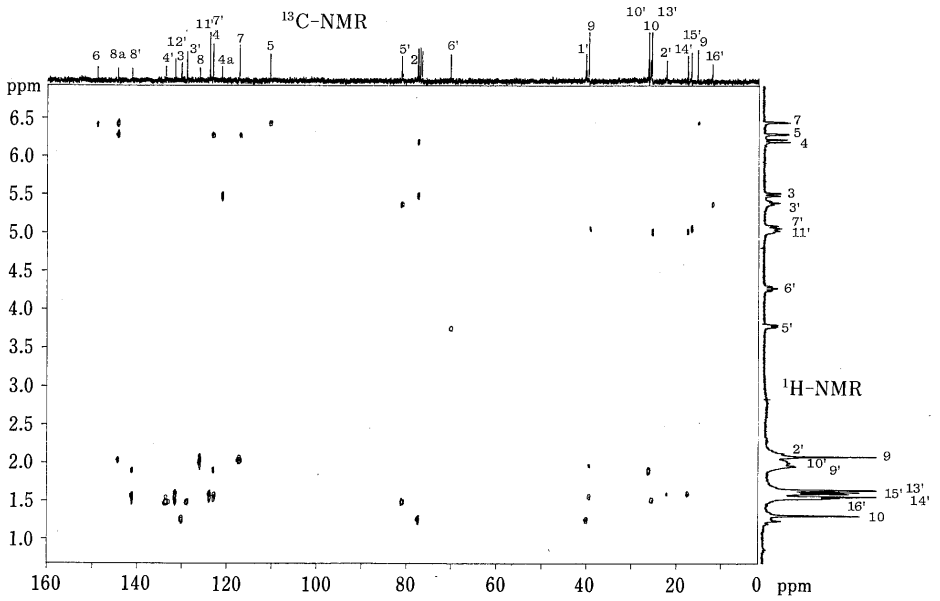

Fig. 1. The XCORFE Spectrum of Sargatriol (**2**)

TABLE I. Cytotoxicity of Partition Fractions of Algal Extracts toward P-388 Cells

Marine algae	ED_{50} (μg/ml)		
	Hexane fr.	CCl_4 fr.	$CHCl_3$ fr.
Brown algae			
Cystophyllum sisymbrioides	22.0	43.5	>100
Hizikia fusiforme	>100	>100	>100
Ecklonia cava	>100	>100	48.2
S. ringgoldianum	>100	>100	26.9
Padina arborescens	>100	>100	48.0
S. tortile	18.0	3.6	7.1
S. thunbergii	>100	13.6	5.6
Eisenia bicyclis	>100	46.5	>100
Dictyopteris prolifera	>100	>100	38.1
Undaria pinnatifira	>100	32.7	36.0
Laminaria japonica	>100	>100	98.8
S. hemiphyllum	>100	56.0	23.3
S. piluliferum	>100	>100	>100
Colpomenia sinuosa	>100	30.6	23.5
Red algae			
Gelidium amansii	>100	18.0	8.2
Scinaia japonica	13.0	>100	30.0
Amphiroa ephedraea	>100	30.5	>100
Schizymenia dubyi	>100	>100	>100
Acrosorium yendoi	25.0	14.0	>100
Chondrus ocellatus	>100	22.3	>100
Carpopeltis flabellata	>100	19.1	15.2
Gracilaria textorii	>100	22.2	32.2
Pterocladia capillacea	>100	>100	16.2
Green algae			
Ulva pertusa	>100	30.6	23.5

Cytotoxicity of etoposide as standard was ED_{50} 0.2 μg/ml.

TABLE II. ^{13}C-NMR Chemical Shifts (δ ppm) of **1**—**4**[a]

Position	**1**	**2**		**3**		**4**
1	187.93					
2	148.65	77.65		77.70		77.67
3	132.24	130.27	130.13	130.30	130.24	129.54
4	188.47	123.11		123.33		123.93
4a		121.17		121.01		120.72
5	133.14	110.49		108.86		109.03
6	146.25	148.97		152.96		153.14
7	15.82	117.30		116.10		116.33
8		126.12		126.14		126.17
8a		144.43		144.85		144.64
9		15.45		15.63		15.66
10		25.79	25.91	25.94	26.01	26.25
1′	27.48	40.24		40.35		39.49
2′	118.36	22.37		22.38		24.14
3′	139.63	129.03		128.75		154.63
4′	39.06	133.72	133.65	133.89	133.96	139.32
5′	25.60	81.15		81.04		195.27
6′	127.94	70.25		70.19		9.10
7′	134.71	123.18		123.33		
8′	80.79	141.32		141.19		
9′	70.11	39.59		39.62		
10′	123.47	26.35		26.39		
11′	141.01	123.86		123.88		
12′	39.71	131.61		131.66		
13′	26.47	25.69		25.69		
14′	123.92	17.68		17.70		
15′	131.65	16.86		16.88		
16′	25.68	12.20	12.00	12.28	12.22	
17′	17.74					
18′	16.89					
19′	12.53					
20′	16.05					
OCH_3				55.66		55.69

a) The data was taken in $CDCl_3$ at 75.4 MHz.

assymmetric centers (C-8′ and C-9′). Assignments of the ^{1}H- and ^{13}C-NMR spectra of **1** and **2** were based on the application of the ^{1}H-homonuclear decoupling technique and ^{1}H–^{13}C heteronuclear shift-correlated (HETCOR) and X-nucleus-proton correlation with fixed evolution time (XCORFE)[4] (Fig. 1) 2D-NMR spectra. Appearance of only one methyl resonance above 20 ppm in the ^{13}C-NMR spectra of **1** and **2** supports the theory that the prenyl side-chain double bonds are all-*E*-oriented.[5]

Compounds **1** and **2** exhibited significant and moderate cytotoxicities (ED_{50} 1.2 and 18.0 μg/ml) against P-388 cultured cells, respectively. However, these compounds did not demonstrate significant activity against the *in vivo* murine P-388 lymphocytic leukemia test system.

Experimental

CD curves were recorded on a JASCO J-500A Spectrometer. Other spectral measurements were carried out with the instruments described in the previous paper.[4]

Extraction and Fractionation of Marine Algae Each fresh alga, collected in May 1985 at the coast of Tanabe Bay, was finely cut and extracted three times with boiling MeOH. The combined extracts were evaporated *in vacuo*. The residue was successively partitioned between MeOH–H_2O (9 : 1) and hexane, MeOH–H_2O (8 : 2) and CCl_4, and MeOH–H_2O (1 : 1) and $CHCl_3$. Removal of the solvents gave hexane, CCl_4, $CHCl_3$ and MeOH–H_2O fractions.

Cytotoxic Activity Evaluation of cytotoxic activity for test material was made according to the procedure previously reported.[4]

Separation of Extract from *S. tortile* A second collection of *S. tortile* (8.8. kg) in Oct. 1985 was extracted with aqueous MeOH, and the resulting extract (586 g) was partitioned according to the procedure described above to afford hexane (58.9 g), CCl_4 (60.8 g), $CHCl_3$ (29.0 g) and MeOH–H_2O (430 g) fractions. The CCl_4 fraction (60 g) was passed through a Sephadex LH-20 column using MeOH–$CHCl_3$ (1 : 1) as the eluent. The cytotoxic activities of the resulting fifth, sixth and seventh fractions were evaluated as ED_{50} 13.7, 3.4 and 18.5 μg/ml, respectively. The sixth fraction (18 g) was repeatedly chromatographed on a silica gel column with a MeOH–$CHCl_3$ gradient as the eluent. Elution with 1% MeOH in $CHCl_3$ and 2% MeOH in $CHCl_3$ gave **1** (500 mg) and **2** (6 g), respectively.

Dihydroxysargaquinone (1) A pale brown oil, $[\alpha]_D^{22}$ +2.1° (c=0.95, $CHCl_3$). High resolution mass spectrum (HR-MS) m/z: 426.2774 (M^+) (Calcd for $C_{27}H_{38}O_4$: 426.2771). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm: 1.59 (6H, s, 17′, 20′-H), 1.61 (3H, s, 19′-H), 1.67 (6H, s, 16′, 18′-H), 2.02 (2H, m, 12′-H), 2.06 (2H, m, 13′-H), 2.07 (3H, d, J=1.1 Hz, 7-H), 2.13 (2H, m, 4′-H), 2.18 (2H, m, 5′-H), 2.43 (1H, s, OH), 3.13 (2H, br d, J=8 Hz, 1′-H), 3.16 (1H, s, OH), 3.85 (1H, d, J=8 Hz, 8′-H), 4.30 (1H, t, J=8 Hz, 9′-H), 5.07 (1H, t, J=7 Hz, 14′-H), 5.14 (1H, d, J=8 Hz, 10′-H), 5.15 (1H, t, J=8 Hz, 2′-H), 5.39 (1H, t, J=6 Hz, 6′-H), 6.49 (1H, dt, J=2.5, 1.6 Hz, 3-H), 6.56 (1H, dq, J=2.5, 1.1 Hz, 5-H). The ultraviolet (UV) and infrared (IR) spectral data of this compound were in accord with the published values.[2b]

Salgatriol (2) A pale brown oil, $[\alpha]_D^{22}$ +15.4° (c=1.0, $CHCl_3$). HR-MS m/z: 426.2772 (M^+) (Calcd for $C_{27}H_{38}O_4$: 426.2771). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.34, 1.35 (3H, each s, 10-H), 1.57, 1.58 (3H, each br s, 16′-H), 1.59 (3H, s, 14′-H), 1.648, 1.653 (3H, each s, 15′-H), 1.67 (3H, s, 13′-H), 1.69 (2H, m, 1′-H), 1.98 (2H, m, 9′-H), 2.03 (2H, m, 10′-H), 2.12 (3H, s, 9-H), 2.15 (2H, m, 2′-H), 2.40 (1H, br s, OH), 3.80, 3.81 (1H, each d, J=8 Hz, 5′-H), 4.29, 4.30 (1H, each dd, J=9, 8 Hz, 6′-H), 4.70 (1H, s, OH), 5.05 (1H, br t, J=6 Hz, 11′-H), 5.11 (1H, br d, J=9 Hz, 7′-H), 5.43 (1H, br t, J=7 Hz, 3′-H), 5.54, 5.55 (1H, each d, J=10 Hz, 3-H), 6.246, 6.251 (1H, each d, J=10 Hz, 4-H), 6.31 (1H, d, J=3.5 Hz, 5-H), 6.47 (1H, d, J=3.5 Hz, 7-H). CD (c=5.54 × 10^{-4} mol/l, MeOH, 20 °C): $\Delta\varepsilon$ (nm): 0 (382), −0.40 (327), −0.19 (290), −0.28 (271), −0.89 (265), −0.70 (247), −2.26 (221), 0 (214). The UV and IR data of this compound were in accord with the published values.[2a]

Sargatriol Methyl Ether (3) Methylation of **2** (91 mg) with diazomethane followed by chromatography on silica gel under elution with 0.5% MeOH in CH_2Cl_2 yielded methyl ether **3** (43 mg) as a pale brown oil, $[\alpha]_D^{26}$ +17.6° (c=0.17, EtOH). HR-MS m/z: 440.2906 (M^+) (Calcd for $C_{28}H_{40}O_4$: 440.2924). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.350, 1.354 (3H, each s, 10-H), 1.57 (3H, br s, 16′-H), 1.58 (3H, s, 14′-H), 1.63, 1.65 (3H, each d, J=1.3 Hz, 15′-H), 1.67 (3H, s, 13′-H), 1.70 (2H, m, 1′-H), 1.98 (2H, m, 9′-H), 2.03 (2H, m, 10′-H), 2.14 (2H, m, 2′-H), 2.15 (3H, s, 9-H), 2.40 (1H, br s, OH), 3.73 (3H, s, OCH_3), 3.79, 3.80 (1H, each d, J=7.6 Hz, 5′-H), 4.27, 4.28 (1H, each dd, J=8.8, 7.6 Hz, 6′-H), 5.05 (1H, br t, J=7 Hz, 11′-H), 5.09 (1H, br d, J=8.8 Hz, 7′-H), 5.42 (1H, br t, J=7 Hz, 3′-H), 5.55, 5.56 (1H, each d, J=10 Hz, 3-H), 6.287, 6.292 (1H, each d, J=10 Hz, 4-H), 6.38 (1H, d, J=3 Hz, 5-H), 6.55 (1H, d, J=3 Hz, 7-H).

Preparation of Aldehyde 4 Oxidation of **3** (40 mg) with a chromium trioxide–pyridine complex in the usual way, followed by chromatography on silica gel under elution with CH_2Cl_2, gave aldehyde **4** (12 mg) as a colorless oil. HR-MS m/z: 286.1568 (M^+) (Calcd for $C_{18}H_{22}O_3$: 286.1568). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.40 (3H, s, 10-H), 1.70 (3H, s, 6′-H), 1.85 (2H, m, 1′-H), 2.16 (3H, s, 9-H), 2.51 (2H, m, 2′-H), 3.74 (3H, s, OCH_3), 5.56 (1H, d, J=9.8 Hz, 3-H), 6.34 (1H, d, J=9.8 Hz, 4-H), 6.40 (1H, d, J=3 Hz, 5-H), 6.51 (1H, br t, J=6.5 Hz, 3′-H), 6.57 (1H, d, J=3 Hz, 7-H), 9.36 (1H, s, 5′-H).

Transformation of 1 to 2 A solution of **1** (15 mg) in pyridine was heated at 50 °C for 48 h and evaporated *in vacuo*. The residue was chromatographed on silica gel and eluted with 5% MeOH in $CHCl_3$ to give **2** (8 mg).

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